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Serial Measurement of Serum Interleukin-2 Receptor Levels in Patients with Rheumatoid Arthritis: Limited Evidence for a Role of T Cell Activation in Clinical Exacerbations

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To investigate the association of T cell activation with clinical exacerbations of RA, we measured serum levels of soluble interleukin-2 receptors (sIL2R), a marker of T cell activation, in serial samples obtained from 23 patients with RA. sIL2R measurements were performed on sera obtained from each patient every 2 weeks for up to 60 weeks, and levels were correlated with swollen joint counts, tender joint counts, physician global assessments, patient global assessments, pain scores, Health Assessment Questionnaire Disability Index scores, and Westergren erythrocyte sedimentation rates measured simultaneously. There were no significant correlations between changes in sIL2R levels and changes in any of the other measures, nor were lead-lag relationships detected, for the group as a whole. Examination of the time courses of individual patients revealed significant positive correlations between changes in sIL2R levels and changes in swollen joint counts in five patients; significant correlations with other measures were present in three or fewer patients. sIL2R levels also varied little over the 2-week time interval of greatest clinical change in each patient. These results suggest either that clinical exacerbations of RA are not associated with changes in T cell activation or that sIL2R levels do not accurately reflect such changes.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of synovial joints. While the etiology of this disease is unknown, an important role for T cells is suggested by the association of RA with particular major histocompatibility complex (MHC) Class II antigens and by the requirement for T cells for the induction of inflammatory arthritis in certain animal models (1, 2). However, it is less clear if T cells mediate chronic synovitis, or if other cell types in the synovium perpetuate inflammation (3). While T cells from synovial tissue of patients with RA display some features of activation, including surface expression of HLA-DR antigen (4–8), other indicators of activation, such as cellular proliferation and response to interleukin-2, are either absent or only minimally enhanced (4–13). Furthermore, although activated lymphocytes would be expected to secrete cytokines, cytokine production by rheumatoid synovial T cells is limited (14–22). Studies of T cell-directed therapies have also failed to clarify the role of T cells in chronic synovitis (23–28). Therapy targeting $CD4^+$ lymphocytes, which accumulate in rheumatoid synovium, has resulted in only limited clinical improvement in the unblinded trials reported to date (29–33).

The role of T cell activation in the pathogenesis of RA has also been investigated by studying the concentration of soluble interleukin-2 receptors (sIL2R) in serum and synovial fluid. IL2R are expressed on the surface of T cells during activation and mediate the autocrine and paracrine effects of interleukin-2 (34). These receptors are shed by activated cells and are detectable in soluble form; higher concentrations have been considered to indicate more intense T cell activation (35, 36). Although T cells in rheumatoid synovium often lack increased expression of surface IL2R (4–8, 11, 13, 14), sIL2R concentrations have been consistently found to be elevated in the serum and synovial fluid of patients with RA, suggesting ongoing T cell activation (37–47).

In several cross-sectional studies, serum concentrations of sIL2R were correlated with the activity of patient's arthritis. However, the relationship between T cell activation and joint inflammation in RA may be more clearly revealed by examining the relationship between changes in sIL2R levels and changes in arthritis activity over time. Although such studies have been reported (37, 38, 41, 48–56), their results have been inconsistent, related perhaps to varying study durations, infrequent patient assessments, or the failure to correlate sIL2R levels with specific clinical indicators of inflammation.

In this time-intensive longitudinal study, we examined the relationship between sIL2R levels and seven

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measures of arthritis activity in 23 patients with RA who were assessed every 2 weeks for up to 60 weeks. Changes in sIL2R levels were not correlated with changes in clinical arthritis activity, suggesting that changes in T cell activation, as reflected by sIL2R levels, do not accompany clinical exacerbations of RA.

METHODS

Patients. The patients in this study were participants in a prospective longitudinal study of changes in RA activity over time, the primary goal of which was to determine the relative accuracy and sensitivity to change of 14 different measures of arthritis activity (57). All participants fulfilled the American Rheumatism Association revised criteria for the classification of rheumatoid arthritis (58), were older than age 18, had the onset of arthritis after age 16, and had evidence of active arthritis, defined as at least six swollen or tender joints, within 4 weeks of study entry.

Twenty-four patients (22 women, 2 men) entered the study (Table 1). The median age of the cohort was 46 years (range 28–73 years), and the median duration of RA was 3.0 years (range 0.2–20 years). Seventeen patients (71%) were seropositive for rheumatoid factor with titers of 1:80 or greater, and 15 of 19 patients (79%) with radiographs available for review had bony erosions. At study entry, 15 patients were taking nonsteroidal anti-inflammatory drugs and 8 were taking low dose prednisone. In addition, 9 patients were being treated with hydroxychloroquine, 5 with methotrexate, 3 with azathioprine, 3 with sulfasalazine, 2 with D-penicillamine, and 1 with parenteral gold. One patient had elected treatment with analgesics alone out of fear of side effects of other medications.

Study protocol. Patients were assessed every 2 weeks for a planned duration of 60 weeks (30 assessments). At each assessment, patients had a joint examination, completed a questionnaire about their symptoms, and underwent tests of functional ability and phlebotomy. Aliquots of serum obtained at each assessment were frozen at −70°C for later testing of sIL2R levels.

Eighteen patients (75%) completed the 60-week study, 1 patient completed 46 weeks, 2 patients completed 40 weeks, 1 patient completed 32 weeks, and 2 patients completed 16 weeks. The group had a total of 635 assessments. Over the course of observation, most patients experienced substantial changes in arthritis activity. For example, each patient had at least a 50% change from baseline value in at least six different arthritis activity measures, and each patient had at least a 30% change from baseline values in nine different arthritis activity measures (57). These changes in clinical status represented both spontaneous changes in arthritis activity and medication-related improvements. Treatment was not provided as a part of this

TABLE 1
Patient Characteristics[a]

Patient	Age/sex	Duration of RA (years)	Rheumatoid factor titer	Medications at study entry	Medications added during study
1	28/F	1.5	1:20	NSAID, SSZ, HCQ, Pred	—
2	53/F	1.0	1:80	NSAID, HCQ	MTX
3	45/F	2.5	Negative	NSAID, MTX, AZA, HCQ	—
4	42/F	9.0	1:640	—	NSAID
5	42/F	4.0	1:80	—	NSAID, Pred
6	67/F	14.0	1:5120	MTX, Pred	—
7	47/F	5.0	1:80	NSAID, DPEN, HCQ	—
8	39/F	13.0	Negative	NSAID, HCQ	—
9	41/F	0.2	1:40	NSAID, HCQ	—
10	54/F	1.5	1:5120	NSAID, Pred	MTX
11	53/F	0.5	1:640	SSZ, MTX	AZA
12	57/M	2.0	1:640	NSAID, Pred	MTX, HCQ
13	47/F	2.5	1:320	NSAID, AZA, Pred	—
14	57/F	10.0	Negative	NSAID	—
15	64/M	10.0	Negative	NSAID	Pred
16	52/F	4.0	1:640	NSAID, AU, HCQ	—
17	73/F	20.0	1:320	SSZ, AZA, Pred	MTX
18	28/F	11.0	1:320	NSAID, MTX, Pred	—
19	52/F	2.0	1:640	NSAID, HCQ	MTX
20	38/F	3.0	1:80	—	—
21	48/F	0.5	1:5120	HCQ, Pred	MTX, DPEN
22	46/F	13.0	Negative	NSAID	—
23	44/F	14.0	1:320	NSAID, DPEN	—

[a] The patient with markedly elevated sIL2R levels is not included in this table. NSAID, nonsteroidal anti-inflammatory drug; SSZ, sulfasalazine; HCQ, hydroxychloroquine; MTX, methotrexate; AZA, azathioprine; DPEN, D-penicillamine; AU, parenteral gold; Pred, prednisone.

study, and patients were free to take whatever medications their physicians prescribed.

Of 14 different measures of arthritis activity assessed at each examination, the tender joint count and the physician global assessment were found to be the most accurate physician-determined measures of arthritis activity, the patient global assessment and pain score were the most accurate patient-determined measures of arthritis activity, the Health Assessment Questionnaire Disability Index (59) was found to be the most accurate functional measure, and the Westergren erythrocyte sedimentation rate (WESR) was the most accurate of three laboratory measures tested (57). Therefore, these measures were used as standards against which changes in sIL2R levels were compared. Because some studies have also reported associations between sIL2R levels and the swollen joint count (37, 45, 49, 52), we also included this measure as a standard. Swollen and tender joint counts were each scored as the number of 50 peripheral joints examined that demonstrated either swelling or tenderness to palpation. The physician and patient global assessments and pain scores were based on 15 cm visual analog scales.

sIL2R Measurements. sIL2R levels were measured in freshly thawed samples using a commercially available enzyme-linked immunosorbent assay (ELISA) (T Cell Sciences, Cambridge, MA). sIL2R concentrations were based on comparison of sample values with a standard curve generated from a standard provided by the manufacturer and were expressed as U/ml. To avoid inter-assay variability, all samples from one patient were assayed together on the same ELISA plate. The intra-assay coefficient of variation was 6.4%. To confirm previous reports of stability of sIL2R levels during prolonged storage (37), an independent set of eight serum samples was tested soon after phlebotomy and again after approximately 18 months of storage; the correlation between measurements was 0.80. As previously reported, sIL2R levels in 26 healthy individuals averaged 462 ± 151 U/ml using this assay (55).

sIL2R measurements were performed on 622 samples. Sixteen patients had no missing samples, but samples were missing for 13 assessments in eight patients. Missing values were replaced with the average of the values that preceded and followed them for each patient. One patient, a 36-year-old woman with seropositive, erosive RA of 2 years duration, had persistent marked elevation of sIL2R levels (all >20,000 U/ml). Because of these abnormally high values and their lack of variation over time, we did not include this patient's data in the analysis. Over the subsequent 3 years, this patient has not developed any other clinical condition that could account for these high sIL2R levels.

Statistical analysis. Correlations between changes in sIL2R levels and changes in each of the seven other measures of arthritis activity over time were estimated using pooled time series regression analysis (fixed effects models) (60, 61). These regression models estimate the within-person correlation between measures while adjusting for differences among patients in characteristics such as age, duration of RA, baseline levels of arthritis activity, and medications. These correlations are, therefore, partial correlations, having been adjusted for between-patient differences. The statistical significance of these partial correlations was based on the associated t statistics of the regression models. To determine if changes in sIL2R might precede or follow changes in the other arthritis activity measures, we also computed correlations between measures lagged by 2 weeks and 4 weeks.

RESULTS

sIL2R levels varied considerably, from 442 U/ml to 19,135 U/ml, among patients at the start of the study (Table 2). Individual patients also demonstrated wide variations in levels over the course of the study. Nineteen of the 23 patients had greater than a 2-fold difference in sIL2R levels, and on average, patients had a 3.3-fold difference in levels, during the study. sIL2R levels were also quite variable over short periods of time. The median maximum change in sIL2R level over 2 weeks was 881 U/ml, and the largest change over a 2-week interval was 8,230 U/ml.

To determine if sIL2R levels were associated with the degree of joint inflammation, we examined correlations between changes in sIL2R levels and changes in swollen joint counts, tender joint counts, physician global assessments of arthritis activity, patient global assessments of arthritis activity, pain scores, functional disability as measured by Health Assessment Questionnaire Disability Index, and WESR in these 23 patients (Table 3). sIL2R levels were not correlated with any of these measures of arthritis activity in this group of patients. All of the partial correlation coefficients were small and none was statistically significant. Similar results were obtained when we examined only the data of the four patients not treated with disease-modifying anti-rheumatic medications or corticosteroids during the study (Table 3).

These findings indicate that sIL2R levels do not vary synchronously with other measures of arthritis activity. To examine the possibility that changes in sIL2R might precede changes in clinical arthritis activity, we correlated sIL2R levels with values of the other arthritis activity measures obtained 2 weeks later in each patient. These 2-week lagged correlations demonstrated little relationship between sIL2R levels and subsequent arthritis activity (Table 3). There was a weak, but statistically significant, negative correlation between the sIL2R level and the tender joint count measured 2 weeks later, suggesting that a decrease in the sIL2R level preceded a small increase in the number of tender joints. Similar results were obtained

TABLE 2
sIL2R Levels for Each Patient during the Study

Patient	Number of assessments	sIL2R (U/ml)			
		Initial	Minimum	Maximum	Maximum 2-week interval change
1	30	1203	304	1465	881
2	30	587	444	1090	415
3	30	1079	510	1841	969
4	30	904	329	2281	1430
5	30	2800	425	3271	2672
6	30	1613	679	2087	1167
7	30	526	390	909	418
8	30	1641	359	1929	1432
9	30	803	353	1199	659
10	30	1457	294	2005	1112
11	30	19135	4535	19980	8230
12	30	1633	1361	2873	1246
13	30	773	464	1024	429
14	30	1799	1081	1943	703
15	30	1681	848	2350	1304
16	20	1020	623	2084	493
17	30	442	406	1418	365
18	16	2947	688	8015	5904
19	30	1879	1017	1908	635
20	20	844	631	2204	1282
21	21	748	495	1066	471
22	8	2777	2392	3015	623
23	8	910	596	1007	375
Group median		1203	510	1943	881
Group mean (Standard deviation)		2139 (3774)	836 (930)	2911 (3989)	1444 (1883)

when we examined a 4-week lag between sIL2R levels and the other measures (data not shown). We also explored whether changes in sIL2R levels might lag behind changes in other arthritis activity measures. Again, these relationships were weak and generally not significant (Table 3).

Although sIL2R levels were not correlated with arthritis activity in the group as a whole, a relationship might be present in individual patients. Therefore, we determined correlations between changes in sIL2R levels and changes in the other arthritis activity measures for each patient individually (Table 4). In five patients (patients 1, 6, 7, 10, and 14), changes in sIL2R levels showed significant positive correlations with changes in the swollen joint count. In 17 other patients, sIL2R levels were not correlated with the swollen joint count, and in one patient, these measures were negatively correlated. No clinical features distinguished

TABLE 3
Correlations (r) of Changes in sIL2R Levels and Changes in Other Arthritis Activity Measures[a]

Measure	All patients		Four patients not treated with DMARDs or steroids		sIL2R precede other measures by 2 weeks		sIL2R follow other measures by 2 weeks	
	r	P	r	P	r	P	r	P
Swollen joint count	−.01	.63	−.21	.63	−.03	.75	−.003	.92
Tender joint count	−.10	.45	−.26	.99	−.10	.02	−.11	.03
Physician global assessment	−.07	.25	−.16	.75	−.07	.10	−.07	.19
Patient global assessment	−.07	.91	−.20	.25	−.07	.80	−.08	.47
Pain score	−.09	.88	−.23	.36	−.08	.46	−.08	.53
Disability index	−.18	.17	.11	.16	−.19	.55	−.18	.79
WESR	−.12	.28	−.29	.37	−.12	.70	−.13	.94

[a] r = partial correlations. DMARD, disease-modifying anti-rheumatic drug.

TABLE 4

Numbers of Patients with Significant Positive Correlations, No Correlations, or Significant Negative Correlations between Changes in sIL2R Levels and Changes in Other Arthritis Activity Measures

Measure	Significant positive correlation	No correlation	Significant negative correlation
Swollen joint count	5	17	1
Tender joint count	2	19	2
Physician global assessment	1	21	1
Patient global assessment	1	22	0
Pain score	1	22	0
Disability Index	3	17	3
WESR	3	19	1

those patients who demonstrated positive correlations between these measures and those who did not. The sIL2R level and the tender joint count were positively correlated in only two patients (patients 2 and 17), neither of whom demonstrated significant correlations between sIL2R and the swollen joint count. One patient each had a significant positive correlation between sIL2R levels and the physician global assessment (patient 2), the patient global assessment (patient 13), and pain scores (patient 13). Patients 13, 17, and 18 had positive correlations between sIL2R levels and the scores on the Health Assessment Questionnaire Disability Index; three other patients had significant negative correlations. Positive correlations between changes in sIL2R levels and changes in WESR were present in three patients (patients 2, 9, and 18). For most patients, however, there were no detectable relationships between sIL2R levels and other measures of arthritis activity.

Although these analyses suggest little correlation between changes in sIL2R levels and arthritis activity over a long period of observation, important short-term relationships during episodes of marked change in arthritis activity might be present, but be obscured by random variation in sIL2R measurements occurring during periods of relatively stable arthritis activity. To examine this possibility, we calculated the changes in sIL2R levels associated with the largest 2-week interval increase (representing worsening) in the physician global assessment and the patient global assessment for each patient (Fig. 1). During time periods in which the physician global assessment increased by a mean of 18 points (on a scale of 0–100), the sIL2R levels increased an average of only 125 U/ml. During time periods in which the patient global assessment increased by a mean of 33 points (on a scale of 0–100), the sIL2R decreased by an average of 7 U/ml. In 12 of the 23 patients, the sIL2R level decreased despite a marked increase in arthritis activity. Therefore, changes in

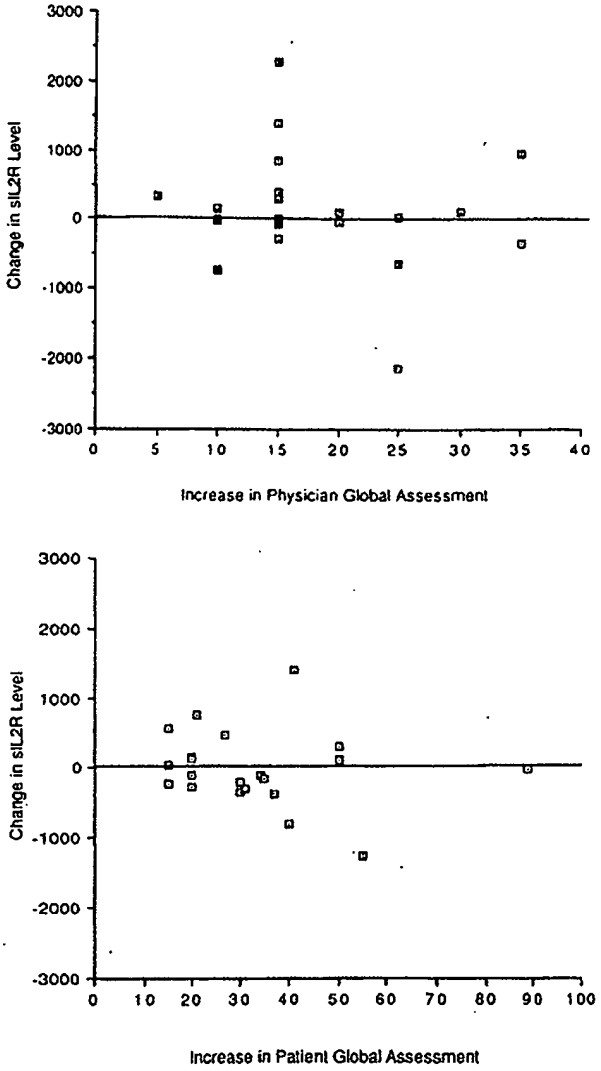

FIG. 1. Relationship between changes in sIL2R levels and the maximal 2-week interval increase in physician global assessment (top) or patient global assessment (bottom) for each patient. Both the physician and patient global assessments are scored from 0 to 100, with higher scores indicating more active arthritis.

sIL2R levels did not appear to be associated with large, short-term changes in arthritis activity in these patients.

DISCUSSION

Among patients in this study, changes in sIL2R levels were not associated with changes in other measures of arthritis activity when assessed every 2 weeks for periods up to 60 weeks. sIL2R levels also varied little during flares of arthritis. In contrast to other studies (38, 48), we were unable to demonstrate that changes

in sIL2R levels preceded changes in clinical status, despite time-intensive monitoring of sIL2R levels.

Several previous longitudinal studies have reported high correlations between sIL2R levels and arthritis activity in patients with RA (37, 38, 41, 48, 49, 52). Notably, Wood and colleagues reported correlations of greater than 0.5 between sIL2R levels and scores on the Ritchie Articular Index, the duration of morning stiffness, pain scores, physician global assessments, and erythrocyte sedimentation rates in 14 patients observed over 1 month (48). Harrington and co-workers reported a correlation of 0.59 between changes in sIL2R levels and changes in the average degree of joint swelling (rather than the swollen joint count) in 14 patients followed for 3 months in a study with a design similar to our own (52). More modest associations between sIL2R levels and joint counts were reported by Rubin and co-workers, who noted a correspondence between the direction of change in sIL2R levels and large improvements in the tender joint count; associations with other measures were not detected (49).

sIL2R levels also decreased approximately 30% in patients who responded to treatment with methotrexate in one clinical trial (54), suggesting a general association between sIL2R and arthritis activity; similar associations, however, were not found in four other clinical trials examining serial sIL2R levels in a total of 229 patients (51, 53, 55, 56). Some of these studies might have failed to detect a relationship between sIL2R levels and changes in clinical status because of short durations of follow-up, or because sIL2R levels may not have been monitored frequently enough. Studies that examine sIL2R levels among patients in clinical trials or after the institution of specific treatments focus on whether clinical improvement is accompanied by decreases in sIL2R levels. By not examining exacerbations as well as improvements in arthritis activity, these studies may have had less opportunity to detect clinical associations with sIL2R levels. Our study overcomes these potential problems by measuring sIL2R levels and assessing arthritis activity frequently in a group of patients over an extended period of time, and by capturing exacerbations as well as improvements in arthritis activity. That the results of our study and those based on clinical trials are similar suggests that the findings of the trial-based studies are not due to these methodological limitations.

If sIL2R levels accurately reflect the degree of T cell activation in patients with RA, our results suggest that changes in T cell activation are not directly associated with clinically detectable changes in joint inflammation. These clinical changes may be more closely related to other inflammatory mediators in the rheumatoid joint (3). It is possible, however, that sIL2R is not the best measure of T cell activation, and examination of other measures of T cell activation may have produced different results. Our findings also do not exclude the possibility that ongoing activation of small numbers of T cells is responsible for driving continued inflammation through their effects on other synovial cells (4). Measurement of sIL2R in serum may not be sensitive enough to detect changes in synovial T cell activation status that, while perhaps occurring in only a limited number of cells, is still sufficient to perpetuate inflammation in the joint. We have previously reported that sIL2R levels do not increase after immunization with a T cell-dependent antigen, suggesting that T cell activation sufficient to produce a vigorous antibody response is not associated with detectable changes in sIL2R levels (62). Similar discrepancies between the state of synovial T cell activation and sIL2R levels may be present in RA.

The specificity of the relationship between serum levels of sIL2R and synovial T cell activation may also be limited. Activated B cells also express IL2R (63, 64), and may be a source of sIL2R. Although *in vitro* experiments with mononuclear cells from normal individuals suggest that T cells are the predominant source of sIL2R (36), it is not known if this is also true of patients with RA. Higher concentrations of sIL2R in synovial fluid than in serum has suggested that the synovium is the primary source of sIL2R in patients with RA (38, 40, 44, 45, 47), but extra-synovial T cell activation could influence sIL2R levels and obscure changes related to synovial T cell activation. Measurement of sIL2R levels in the serum and synovial fluid of patients receiving T cell-directed immunotherapy would be helpful in determining if the synovium is the primary source of sIL2R in RA. In two studies of anti-CD4 monoclonal antibody therapy in patients with RA, serum levels of sIL2R did not change despite clinical improvement (33, 65), suggesting that sIL2R may be derived from other tissues in addition to the synovium. Levels of sIL2R may also be affected by processes unrelated to arthritis activity, such as infections and other antigen-specific immune responses.

Despite these potential limitations in sensitivity and specificity, sIL2R levels were correlated with changes in the swollen joint count in 5 of the 23 patients studied, indicating that joint inflammation may be related to T cell activation in some patients. The importance of this association in this small number of patients is unclear, but it may reflect pathogenetic heterogeneity in RA. Patients in this subgroup did not share clinical features, with a wide range of ages, arthritis of varying severity, and early as well as long-standing RA represented. The group also included seronegative as well as seropositive patients, suggesting that any common T cell-associated pathogenetic mechanism present among these patients was not associated with similar autoimmune B cell responses. In addition, sIL2R levels were not correlated with other arthritis activity measures in these patients, which might have served to corroborate an association between sIL2R levels and

joint inflammation in this subgroup. In addition, although sIL2R levels were not correlated with changes in clinical arthritis activity, sIL2R levels were elevated in most patients, suggesting some role for T cell activation in RA.

In summary, changes in sIL2R levels were not correlated with changes in joint inflammation over time in this cohort of patients with RA, nor did sIL2R levels change appreciably during periods of rapid change in arthritis activity. To the extent that sIL2R levels reflect T cell activation, changes in T cell activation do not appear to play a role in mediating clinical exacerbations of RA. Moreover, sIL2R does not appear to be a clinically useful measure of arthritis activity in patients with RA.

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